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Nitrogen fixation by native *Bradyrhizobia* in symbiosis with *Lupinus mariae-josephae* requires a T3SS encoding a NopE-like effector

David Duran¹, Victor Pastor¹, Susanne Zehner², Michael Göttfert², Juan Imperial¹, Tomás Ruiz-Argüeso¹, **Luis Rey¹**

¹Departamento de Biotecnología (ETS de Ingenieros Agrónomos) and Centro de Biotecnología y Genómica de Plantas (CBGP). Universidad Politécnica de Madrid, Pozuelo de Alarcón, ES,

²TU Dresden, Institute of Genetics, Dresden, DE

Several bradyrhizobial isolates from *L. mariae-josephae* root nodules [1] contain a type III secretion system (T3SS) within a cluster of about 30 genes. Among those genes, *ttsI* codes for the transcriptional activator of the system. Mutation of *ttsI* resulted in the formation of white, non-fixing nodules with the natural legume host, *L. mariae-josephae*. The T3SS cluster also contains a gene coding for a NopE-like protein. NopE proteins have been demonstrated to be effectors in the *Bradyrhizobium*-soybean symbiosis [2] and belong to a small group of poorly characterized proteins from plant-associated bacteria that contain one or two autocleavage motifs known as DUF1521 (Schirromeister *et al.* 2011). The amino acid sequence of a NopE-like protein in the *L. mariae-josephae* strain LmjC contains just one autocatalytic motif. This is unlike NopE1 and NopE2 proteins secreted by the T3SS of *B. japonicum*, that contain two motifs [3]. The autocleavage of LmjC NopE protein was analyzed after expression in *E. coli* and purification. Two protein fragments of the predicted sizes appeared in the presence of Ca²⁺, Cu²⁺, Cd²⁺, Zn²⁺ and Mn²⁺ cations. In contrast, autocleavage did not take place in the presence of Ni²⁺, Co²⁺ or Mg²⁺. Site-directed mutagenesis of the DUF1521 motif in LmjC NopE abolished self-cleavage in vitro. Symbiotic competence of a NopE⁻ mutant with the *L. mariae-josephae* host was not affected. Possible roles of NopE are discussed.

References

- [1] Duran et al. (2013) Syst Appl Microbiol 36:128-136.
- [2] Wenzel et al. (2010) MPMI 23:124-129.
- [3] Schirromeister et al. (2011). J. Bacteriol. 193:124-129.

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